THE NATURE OF GRAFT-HYBRIDS

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THE possibility of hybrids arising as the result of grafting long has been a mooted point and the subject has given rise to much discussion.

The history of the small number of graft-hybrids that have hitherto been recorded is small and is not as complete as might be wished; indeed it has been claimed repeatedly that these supposed graft-hybrids are not really such but have been produced by the ordinary method of cross-fertilization. The most famous of these graft-hybrids is the much discussed *Cytisus Adami* which originated at Vitry near Paris about 1826. This was said to have been the result of grafting *Cytisus purpureus* upon *C. laburnum*. A series of supposed graft-hybrids is also recorded resulting from grafts between a thorn, *Cratægus monogyna*, and the medlar, *Mespilus germanicus*. Three of these graft-hybrids were secured by Bronvaux. The hybrids in this case were not all alike and were given special names and the genus *Cratægomespilus* was proposed for these bi-generic hybrids.

Of the recent opponents of the graft-hybrid theory the best known is the distinguished botanist Professor E. Strasburger, of Bonn. Strasburger made a careful cytological study of *Cytisus Adami* which has been retained in cultivation ever since its origin some eighty-five years ago. Strasburger came to the conclusion that *Cytisus Adami* was a real sexual hybrid and not a graft hybrid. He believes that if the latter were true the nuclei of the hybrid would show a double number of chromosomes. This, of course, implies that in hybrids arising otherwise than sexually, assuming that a nuclear fusion would precede the formation of such a hybrid, there would be no

reduction division of the nuclei comparable to that which normally occurs before the fusion of the sexual cells in normal fertilization.

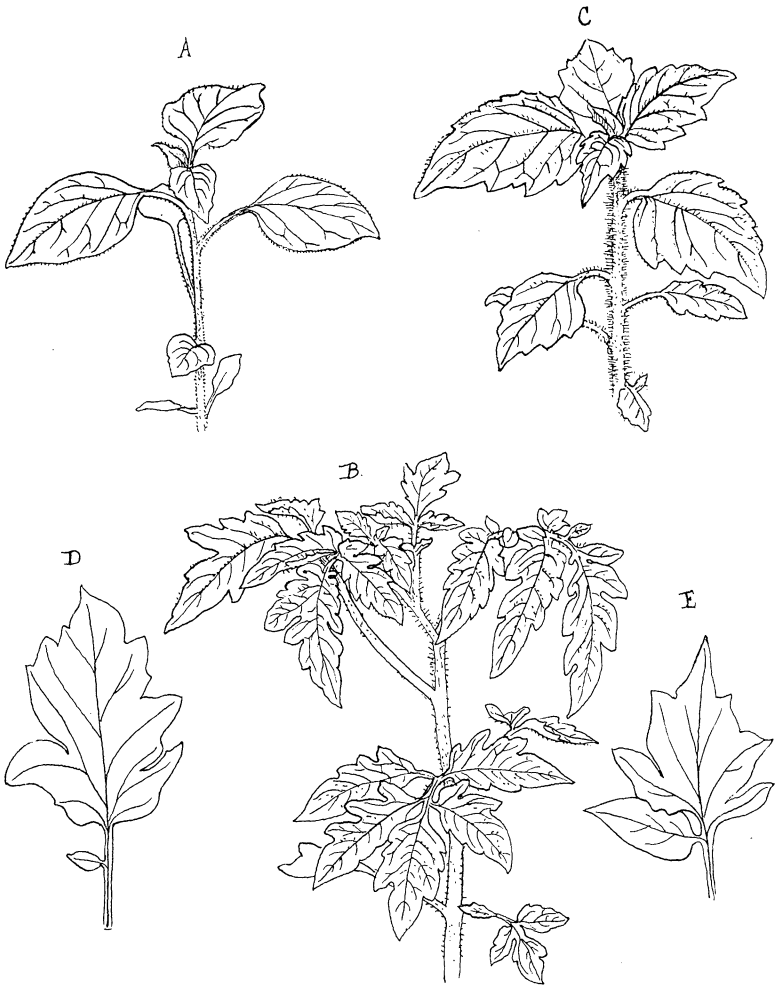

FIG. 1. *A*, seedling of the black nightshade, *Solanum nigrum*; *B*, seedling of a tomato, *S. lycopersicum*; *C*, shoot of the graft-hybrid, *S. tubingense*; *D*, *E*, leaves of the graft-hybrid, *S. proteus*. (All figures after Winkler.)

Němec,[1] however, believes that a reduction division does occur, and there is, therefore, no reason to expect

[1] Němec, B., "Zur Mikrochemie der Chromosomen," *Ber. der deutsch. Botan. Gesellsch.*, 27, 46, 1909.

an increase in the number of chromosomes in the cells of the hybrid. If such a reduction does occur *Cytisus Adami* would show the same number of chromosomes as *C. laburnum* which has the same number as *C. purpureus.*

The study of graft-hybrids has assumed a new interest through the very important recent investigations of Professor H. Winkler, of Tübingen. These investigations prove beyond question that graft hybrids are possible, and the numerous experiments carried out with every possible precaution and showing remarkable ingenuity as well, furnish by far the most important study on the nature and origin of graft-hybrids that has yet been published. These experiments are being further developed by Professor Winkler but the results already obtained are of the greatest interest and value.[2]

The fact that hybrids may arise as a result of grafting touches some of the fundamental problems of heredity, and this makes these papers of Professor Winkler of the highest importance to all students of heredity, and they deserve much wider attention than they have as yet received.

The plants chosen by Winkler for his experiments were the black nightshade, *Solanum nigrum,* and two varieties of the tomato, *Solanum lycopersicum.* These two species are very distinct, and indeed many botanists regard the tomato as belonging to a distinct genus *Lycopersicum,* so that Winkler's graft-hybrids might be regarded as bigeneric like the *Cratægo-mespilus* graft-hybrids.

The methods by which Winkler secured his graft-hybrids were extremely ingenious. Seedlings of the night-

[2] 1. "Uber Propfbastarde und pflanzliche Chimæren," *Ber. d. deutsch. botan. Gesellsch.,* 25, 568–576, 1907.

2. "Solanum tubingense, ein echter Propfbastard zwischen Tomate und Nachtschatten," *ibid.,* 27, 595–608, 1908.

3. "Weitere Untersuchungen über Propfbastarde," *Zeitschr. für Botanik,* 1, 315–345, 1909.

4. "Uber die Nachkommenschaft der Solanum-Propfbastarde und die Chromosomenzahlen ihrer Keimzellen," *ibid.,* 2, 1–38, 1909.

5. "Uber das Wesen der Propfbastarde (Vorläufige Mittheilung)." *Ber. der deutsch. botan. Gesellsch.,* 28, 116–118, 1910.

shade and of the tomato were decapitated and reciprocal grafts were made. In making these unions the graft was cut either wedge-shaped or saddle-shaped at the point of junction with the stock. The graft and stock united readily whether the nightshade or tomato was used as the stock. After the union was complete the plant was again decapitated, the cut being made through the region where the union had taken place. The cut surface thus exposed is composed of tissue derived from the two members of the union and from this cut surface a callus soon develops from which numerous adventitious buds quickly arise. It was thought that from some of these adventitious buds arising at the point of the junction of the graft and stock there might be produced shoots which would combine the characteristics of the two, or at least might be composed of tissue derived from the two parents.

Naturally the great majority of the shoots arising from the cut surface of the stem were either pure nightshade or pure tomato. But finally shoots were observed which were evidently of mixed origin. The first of these graft-hybrids were obviously composed of pure elements derived from the two parents. Some of these shoots were almost equally divided by a median line on one side of which the organs—stem, leaf, etc.—were those of the nightshade, while on the other the organs were evidently derived from the tomato. Sometimes a leaf was nearly equally divided. In most cases one or the other of the parents predominated, but there was no intermediate region between the two kinds of tissues and organs. It is clear that such monstrous forms, for which Winkler proposes the name "chimæra," are not hybrids in any true sense of the word, but have arisen from buds in which there was a mere mechanical coalescence of tissue from the two parent forms at the junction of the stock and graft.

Further experiments, however, resulted in the production of shoots in which the characteristics of the two parents were so intimately combined, that their discov-

erer felt warranted in assuming that these were really hybrids, probably arising from the actual fusion of cells derived respectively from the nightshade and the tomato, this fusion taking place where the graft had united with the stock. This cell-fusion was assumed to involve a fusion of the nuclei as well, analogous to the fusion of the egg-nucleus with the generative nucleus of the pollen tube in normal fertilization.

Several types of these graft-hybrids were produced and to these specific names were given.

The first genuine graft-hybrid was called *Solanum tubingense* and it has since been produced several times and has been propagated by cuttings and distributed to various botanical gardens. During the past summer I had an opportunity of seeing this graft-hybrid growing well in the botanical gardens of the University of Munich.

Solanum tubingense is intermediate in external appearance between the nightshade and tomato but is rather nearer the former (see Fig. 1, *C*). The nightshade (*A*) has simple, smooth-edged, oval leaves and an almost smooth stem. The tomato (*B*) has compound leaves with sharply serrate leaflets and all of the varieties are strongly hairy. The hybrid (see Fig. 1, *C*) has simple leaves but they are sharply serrate or often slightly lobed like the leaflets of the tomato, and both stem and leaves are abundantly provided with hairs.

The flower in *Solanum tubingense* is also intermediate in character. The nightshade has small white flowers with a smooth calyx whose lobes are very short. The flower of the tomato is much larger, bright yellow in color and the lobes of the calyx are hairy and very much longer than those in the nightshade. The hybrid has flowers which are intermediate in character. They are larger than those of the nightshade but much smaller than those of the tomato, but like the latter the flowers are a pronounced yellow. The calyx lobes are two or three times as long as those of the nightshade but much shorter than those of the tomato. Like the latter, how-

ever, there are numerous hairs upon the calyx lobes which in the nightshade are almost smooth.

The fruit of *Solanum tubingense* is very much like that of the nightshade but is rather larger, and although it is black in color there are some traces of the red or yellow color of the tomato.

Four other well-marked graft-hybrids were secured to which were given the names *Solanum proteus, Solanum darwinianum, Solanum koelreuterianum* and *Solanum gaertnerianum.*

The first of these originated in a most peculiar fashion. A chimæra was obtained which consisted of *two* hybrid components. One of these was the before mentioned *S. tubingense* while the other was a hybrid which was more like the tomato. This chimæra soon divided into two branches one of which was pure *S. tubingense* and the other the new hybrid, *S. proteus.* The latter was then removed and rooted and further propagated by cuttings. This species has very variable leaves (see Fig. 1, *D, E*) which on the whole are more divided than those of *S. tubingense,* while in the characters of the flower and the fruit it is more like the tomato than like the nightshade.

Both of the forms *S. koelreuterianum* and *S. gaertnerianum* were produced more than once and they are respectively more like the tomato and nightshade but each differs in important particulars from either of the parents.

The form, however, which is of the greatest interest is the hybrid to which Winkler gave the name *S. darwinianum,* the third to result from his experiments. This hybrid arose in a quite different manner from the others and great ingenuity was shown in isolating and propagating it. The shoot from which this hybrid originated was a chimæra which developed from a graft of a tomato upon a nightshade. This chimæra was made up principally of pure *Solanum nigrum,* but a small portion of it consisted of tissue which was different from any

of the forms which had yet been discovered. The chimæra instead of being made up of two portions united longitudinally was composed mainly of tissue evidently of pure *Solanum nigrum* origin. A small strip, however, near its base was of a different character. This region consisted of a single leaf, and a small amount of tissue lying below belonging to the stem. The same form was secured a second time where it developed from a five-fold chimæra derived from *S. proteus*. Unfortunately, it was not possible to propagate this second specimen.

In order to isolate this new form it was necessary to cause the axillary bud belonging to the single leaf to develop into a shoot. This was finally successful after four decapitations of the *Solanum nigrum* shoot above it. The final result was a branch which was very different from any of the previously developed forms and it was named *Solanum darwinianum*. The point of special interest in connection with this form is that of all graft-hybrids which Winkler secured, this seems to be the only one which is likely to prove a hybrid in the strict sense of the word. This point, however, will be brought out later in the discussion of the real nature of these graft-hybrids.

All of the hybrids were propagated further by cuttings and with the single exception of *Solanum koelreuterianum*, were made to produce ripe fruit which in all cases was more or less intermediate in character between the fruit of the nightshade and the tomato. In *Solanum darwinianum*, however, the fruit was all sterile and no perfect seeds were formed. The fruit itself is a small round berry like the fruit of the nightshade in form, but having the color and structure of the tomato. In *Solanum koelreuterianum* the young fruit set but failed to reach maturity.

Of the hybrids *Solanum tubingense* is the most fertile and produces fruit very abundantly. A considerable number of the fruits, however, are sterile or "parthenocarpic" and the seeds in no cases reach their full de-

velopment. Nevertheless Winkler was able to make these seeds germinate and the second generation of the plants was reared. *S. gaertnerianum* produces fruit only in small numbers but the seeds are perfectly developed and germinate readily, the same being true of *S. proteus*.

REVERSIONS

Winkler observed a number of cases where the graft-hybrid reverted to one or other of the parent forms. Similar cases of reversions have been recorded for *Cytisus Adami* and *Cratægo-mespilus*. These reversions were studied with special care in his first hybrid *S. tubingense*. In several instances where the plant was cut off below the first lateral bud numerous adventitious shoots arose from the cut surface, and while some of these were pure *S. tubingense,* others were pure *Solanum nigrum,* the parent species which is nearer to *S. tubingense*. In a similar manner *S. proteus* was observed frequently to revert to the tomato, but in no case was there reversion to the nightshade.

Sometimes spontaneous reversions occur. Thus in *S. tubingense* the apex of a plant was noted which had suddenly assumed the characters of *S. nigrum*. Winkler gives an excellent photograph of this plant. In other cases shoots of mixed nature were seen, some having the structure of chimæras, half nightshade and half the hybrid form. In these mixed shoots the inflorescence had flowers of two sorts belonging respectively to the nightshade and to the hybrid. Similar mixed inflorescences have also been observed in *Cytisus Adami*.

The Second Generation

In *S. proteus* and *S. gaertnerianum* perfect seed is developed and germinates readily. *S. tubingense* which sets fruit freely never has the seed fully developed but as we have already stated Winkler succeeded in germinating these seeds and rearing plants from them. He

explains the failure of the seeds to develop fully to the fact that the fruit of the hybrid, which closely resembles that of the nightshade, ripens before the seeds have had time to complete their development. The tomato fruit requires a very much longer time for maturing than does the berry of the nightshade and a correspondingly longer time is needed for the seed to be perfected; and he thinks that the longer time required for the seed development in *S. tubingense* is an inheritance from the tomato parent, while the fruit is mainly of nightshade derivation.

All of the seedlings derived from these hybrids reverted absolutely to that parent form which the hybrid more nearly resembles. Thus the seedlings of *S. tubingense* and *S. gaertnerianum* are pure *S. nigrum,* those of *S. proteus* pure tomato. This behavior also corresponds to that of the very few cases where seedlings have been secured from *Cytisus Adami,* these in all cases proving to be pure *Cytisus laburnum.*

Of the *Cratægus-mespilus* hybrids only one, *Cratægo-mespilus asnièresi* produced seed capable of germinating. These seedlings were not reared to maturity but so far as could be judged from the young plants, were pure *Cratægus monogyna,* the parent which the hybrid more nearly resembled.[3]

The third and fourth generations of the *S. tubingense* seedlings retain perfectly the characters of *S. nigrum* and the same is the case when they are cross-pollinated by *S. nigrum.* Attempts to cross *S. tubingense* with the tomato resulted in the formation of fruit but no seeds were developed. It may be also recorded that crosses between the two parent forms, the nightshade and the tomato, were without any result.

S. proteus crossed with the two parent forms produced seed when crossed with the tomato to which it stands the nearer, and sterile fruit when crossed with *S. nigrum.*

[3] Noll, F., "Die Propfbastarde von Bronvaux," *Sitzungsber. der niederrheinische Gesellsch. für Natur- und Heilkunde,* 1905.

As yet no seed has been obtained from crosses between the graft-hybrids themselves.

The Nature of Graft-Hybrids

Winkler concluded at first that all the graft hybrids except the chimæras probably arose from actual cell fusion and might be compared directly with hybrids arising from true fertilizations. It was suggested by another student of graft-hybrids, Bauer,[4] that these apparent true hybrids might also be chimæras of a type which he has called "periclinal," *i. e.*, the outer tissues are derived from one parent, and the inner tissues from the other, but none of the tissues themselves are of hybrid nature. This hypothesis seemed the more probable from the results of investigations of MacFarlane upon *Cytisus Adami*[5] in which he showed that the epidermal tissues were strikingly like those of *C. purpureus* while the inner tissues were like those of *C. laburnum*. An investigation of the *Cratægo-mespilus* hybrids revealed a similar state of affairs.

Acting on this suggestion Winkler made a careful cytological study of his hybrids and found that four of them were indeed periclinal chimæras. But one of them seemed to be a real hybrid resulting apparently from a fusion of cells at the junction of the graft and stock.

The nuclei in the nightshade and the tomato differ very much in the number of the chromosomes so that the determination of the origin of the tissues in the hybrid is made comparatively easy. The chromosome number in the sporophytic tissue is twenty-four in the tomato and seventy-two in the nightshade. These numbers were found in the tissues of all of the graft hybrids except *S. darwinianum* where the reduced number of the

[4] Bauer, Erwin, "Propfbastarde," *Biologisches Centralblatt*, 33, No. 15, 497–514, 1910.

[5] MacFarlane, J. M., "A Comparison of the Minute Structures of Plant Hybrids with those of their Parents, and its Bearing on Biological Problems," *Trans. Roy. Soc. of Edinburgh*, 37, 203–286, 1895.

chromosomes in the germ cells was found to be twenty-four, which was to be expected if these were derived from cells with forty-eight chromosomes; *i. e.*, one-half the number of the twenty-four plus seventy-two chromosomes of the two parents. It is assumed by Winkler that a reduction in the number of chromosomes follows the fusion of the cells. He says:

> This chromosome number, *i. e.*, forty-eight, is most readily explained by the assumption that in the formation of the graft hybrid a nightshade cell (with seventy-two chromosomes in its nucleus) and a tomato cell (with twenty-four chromosomes) united. The resulting cell, from which the subepidermal layer at the apex of the darwinianum hybrid arose, had a nucleus with ninety-six chromosomes which then underwent a reduction division resulting in forty-eight chromosomes.

This study of the tissues of *S. darwinianum* indicates that the subepidermal tissue from which the sporogenous cells develop is of genuine hybrid nature arising from a fusion of cells including the nuclei derived from the two parent forms.

In his latest paper (5) Winkler gives a brief summary of his conclusions which are as follows:

> Hybrids may be arranged in two groups, sexual and graft hybrids. The latter may be divided into three classes according to the theoretical possibility of their method of origin, viz.: (1) Fusion graft-hybrids arising from a fusion of two somatic cells derived from distinct species. (2) "Influenced" ("Beeinflussungs Propfbastarde") graft-hybrids which arise from specific influences of one graft component upon the other without cell fusion (as through chemical substances, translocation of cytoplasm, etc.). (3) Chimæras, in which specifically pure cells from both graft components are combined to form a new individual. These chimæras may be: (*a*) Sectorial chimæras in which the two sorts of cells in the growing point are divided by a longitudinal plane. (*b*) Periclinal chimæras in which the periclinal cell layers of the growing point are furnished respectively from one or the other parent form. (*c*) Hyperchimæras in which the growing point is made up of a mosaic of cells derived from the two parent forms.

The first of Winkler's graft-hybrids were unmistakably chimæras of the first type. Of his later graft-hybrids to which he gave special names, all except *S.*

darwinianum are periclinal chimæras. This is true also of *Cytisus Adami* and the *Cratægo-mespilus* hybrids. Thus *S. tubingense* has its epidermal region derived from the tomato while the inner tissues including those which give rise to the sporogenous cells are of nightshade origin. In *S. proteus* the reverse is the case. This explains all cases of reversion to the parent forms and also the character of the seedlings which in the one case are pure nightshade and in the other tomato, this being due to the fact that the spores (pollen spores and embryo-sacs) arise from sub-epidermal tissues derived from the nightshade or the tomato as the case may be.

These remarkable experiments of Winkler's must be of the greatest interest to all students of the problems of heredity. They emphasize a fact, too often overlooked, that it is not always safe to apply to the study of plants the data of zoology. It must be remembered that in the evolution of the higher plants there has been a constant tendency toward a reduction of the sexual reproductive parts. Many biologists quite ignore the fact that the flowering plant, as it is generally understood, is a purely sexless organism. The so-called sex organs, stamens and carpels, are not such at all, but are non-sexual sporophylls.

The sexual generation of the highest seed plant is a far simpler organism than that of the moss or fern and the sex organs are correspondingly simpler. Moreover the development of the sex cells and the extraordinary correspondences in nuclear structure, the reduction divisions and the mechanics of fertilization must have developed quite independently of these phenomena in animal cells, since the two great divisions of organisms, plants and animals, parted company for good long before the elaborate structures found in the higher members of the two series were developed. Hence it by no means follows that what is true in one case must necessarily follow in the other.

With the subordination of the sexual generation of

the higher plants there goes a high degree of regenerative power, a great contrast to the very limited capacity for regeneration shown by the highly organized animals where new individuals can only arise through sexual reproduction. This great power of regeneration in plants is accompanied by much less specialized cells and a very imperfectly marked individuality of the organism as a whole. Any seed plant may be regarded as a colony of individuals since the parts are repeated indefinitely and can be made to regenerate the whole plant. The power of regeneration shown by almost any part of the plant, even a single cell in some cases, renders any theory of a special germ plasm out of the question in the case of plants, however plausible such a theory may appear when applied to animals.

It is not then so very extraordinary that this regeneration of the plant from somatic cells should be carried so far as to involve cell fusions such as Winkler believes preceded the formation of his *Solanum darwinianum*. Even if this should not be proved, his experiments show beyond question the existence of graft-hybrids of a sort quite inconceivable in any animals except very low types, such as corals where it is by no means impossible that similar graft-hybrids might be developed.

It is this positive demonstration of the reality of "vegetative" or "somatic" hybrids which gives the experiments of Winkler their greatest value, and it is to be hoped that they will serve as a stimulus to other work in the same direction which may well have a great influence upon the drift of biological speculations dealing with the laws of heredity.